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UVB Immunosuppression: Vitamin D or Not Vitamin D? That Is the Question

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UVB radiation stimulates the production of vitamin D and also has immunosuppressive effects. Vitamin D is also known to alter immunological function. Thus, a relevant question is whether vitamin D is a mediator of the immunological effects of UVB. In this issue, Schwarz *et al.* have addressed this issue and have concluded that although vitamin D has similar effects to UVB on the immune system, UVB-induced immunosuppression can be achieved without the requirement for vitamin D action.

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Vitamin D, a molecule formed in the skin following UVB radiation exposure, is a major regulator of calcium homeostasis and an essential nutrient for bone health. Children whose diet lacks vitamin D or who have an inborn error in vitamin D metabolism develop rickets, and adults deficient in vitamin D can develop osteomalacia and osteoporosis. There is unequivocal evidence that vitamin D is necessary for bone health; but, in recent years, there has been great interest in vitamin D's extraskeletal effects. These include protective effects in colon and breast cancer, prevention of cardiovascular disease, diabetes, and pre-eclampsia, and modulation of immunological function. However, it is important to note that the Institute of Medicine has concluded that, at this time, the data in humans are insufficient to conclude that vitamin D has effects other than on bones, highlighting the need for further study in this area (Ross *et al.*, 2011).

The effects of vitamin D on cutaneous immunity are controversial. Some studies have shown that topical application of vitamin D can protect against UV-induced DNA damage and diminish its immunosuppressive effects. In contrast, other studies, including the one by Schwarz *et al.* (this issue, 2012) in this issue of the *Journal of Investigative Dermatology*, have demonstrated that vitamin D facilitates the development of regulatory T cells (Tregs) and is necessary for the production of T cells that produce IL-17 (Th17 cells) and IL-9 (Th9) (Palmer *et al.*, 2011). In support of the concept that vitamin D downregulates immune responses are clinical studies showing that the concentration of vitamin D correlates with the level of Treg in the peripheral blood of patients with multiple sclerosis (Royal Iii *et al.*, 2009). Epidemiologic studies have suggested that individuals with vitamin D deficiency are predisposed to a variety of other autoimmune diseases, including inflammatory bowel disease, systemic lupus erythematosus, multiple sclerosis, and type I diabetes (Arnson *et al.*, 2007). The conflicting observations may result from differences in experimental systems and treatment regimens, the complex mechanisms by which vitamin D can act on innate and adaptive immunity (Hart *et al.*, 2011), or polymorphisms in nuclear or membrane receptors for vitamin D among individuals.

Similar to vitamin D, UVB radiation has been shown to modulate immune function, an observation that was made nearly 40 years ago and that gave rise to the discipline of photoimmunology (Krutmann and Elmets, 1995). The effects of UVB on the immune system help to explain the greatly increased incidence of non-melanoma skin cancer in organ transplant recipients, the efficacy of phototherapy in immunodermatological disorders, and the pathogenesis of a number of photosensitivity disorders such as polymorphous light eruption. After UV radiation exposure, immunosuppressive cytokines are produced and alterations in cutaneous antigen-presenting cells occur, shifting the balance in the T-cell-mediated immune response to one in which regulatory, rather than effector, T cells predominate. The Tregs that develop after the introduction of antigens to UVB-irradiated skin have been well characterized (Elmets *et al.*, 1983; Schwarz, 2008). They are Fox-p3$^+$, and they express the CD4 and CD25 phenotypic markers; they are antigen specific and, once activated, produce the cytokine IL-10, which is, at least in part, responsible for their immunosuppressive activities.

The generation of Tregs by UVB radiation requires dendritic cells (DCs) in the skin. These cells, in most circumstances, are responsible for presentation of antigens to both effector and Tregs. However, after UVB radiation exposure, their antigen-presenting function becomes biased toward the generation of Tregs (Schwarz *et al.*, 2010). The evolutionary rationale for this response to UVB exposure is uncertain, but it may result from efforts to protect against autoimmune diseases such as polymorphous light eruption and lupus erythematosus. There are multiple DC populations in the skin. Two of these populations—epidermal Langerhans cells and dermal langerin+ DCs—have distinct functions in the induction and regulation of cutaneous immune responses. Specifically, epidermal Langerhans cells are required for the development of UVB-induced Tregs; on the other hand, evidence suggests that dermal langerin+ DCs are important for effector T-cell responses.

Speculation on the molecular target for the photoimmunological effects of

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Clinical Implications

- Topical vitamin D analogs work in psoriasis by augmenting regulatory T cells through effects on Langerhans cells.
- Substituting topical vitamin D for phototherapy or combining the two therapies may be effective in selected immunodermatological diseases.
- Vitamin D receptor polymorphisms, at least in part, may explain genetic susceptibility to some immunologically mediated skin diseases.

UVB radiation has centered on DNA damage and its repair, although other molecules, such as platelet-activating factor, *cis*-urocanic acid, and noncoding regions of RNA, have also been implicated (Sreevidya *et al.*, 2010; Bernard *et al.*, 2012). Given the similarity in the effects of vitamin D and UVB radiation on the cutaneous immune system, and the fact that wavelengths within the UVB are responsible for synthesis of vitamin D from 7-deoxycholesterol, it is not surprising for vitamin D to be proposed as an additional mediator of the immunosuppressive effects of UVB radiation. Schwarz *et al.* (2012) have carefully addressed this issue. They evaluated the immunological effects of vitamin D in a well-established system of cutaneous T-cell-mediated immunity. They observed that topical application of 1α, 25-dihydroxyvitamin D_3 can induce immunosuppression with features that mirror those of UVB radiation, but that the mechanism by which immunosuppression is induced appears to be distinct. The findings are consistent with studies in animal models showing that UV radiation suppresses experimental autoimmune encephalomyelitis independent of vitamin D production (Becklund *et al.*, 2010).

Schwarz *et al.* (2012) were able to show that topically applied 1α, 25-dihydroxyvitamin D_3 had an inhibitory effect on the induction of contact hypersensitivity. This effect was mediated by antigen-specific Foxp3$^+$ Tregs. It has been shown that vitamin D alters the function of DCs in the activation of T cells and the induction of cell-mediated immune responses (Hart *et al.*, 2011). Schwarz *et al.* (2012) confirmed that finding; moreover, they showed that Langerhans cells have a major role in both suppression of contact hypersensitivity and in the development of Foxp3$^+$ Tregs. However, UVB-induced immune suppression was not abolished in vitamin D receptor–deficient mice. The inference of this finding is that UV irradiation and vitamin D induce immunosuppression differently.

Further studies will be required to establish whether vitamin D has effects *in vivo* in humans similar to those observed in mice. Vitamin D has been found to regulate the differentiation and function of both epidermal Langerhans cells and dermal DCs in humans (Schauber and Gallo, 2008; Hart *et al.*, 2011) and, when compared with UVB, to induce the development of different types of Tregs (van der Aar *et al.*, 2011).

What are the clinical implications of the study? Topical vitamin D analogs are already used to treat psoriasis, a disease in which an overactive immune response is known to have a pathogenic role. The findings of Schwarz *et al.* (2012) suggest that one mechanism by which topical vitamin D analogs work in psoriasis is through effects on immunity, by augmenting the induction of Tregs, and/or through effects on epidermal Langerhans cells. Consistent with the finding that vitamin D and UVB (which is also used to treat psoriasis) lead to the generation of Tregs through different mechanisms are clinical studies demonstrating that the combination of UVB and topical vitamin D analog treatment can improve therapeutic efficacy in psoriasis beyond that of either alone.

There are anecdotal reports and small series of the use of vitamin D for other immunologically mediated cutaneous diseases, such as morphea and vitiligo. The findings of the current study provide a rationale for expanded clinical trials examining the use of topical vitamin D in these and other diseases for which current therapy is suboptimal.

UVB radiation has many different effects on the skin (such as damage to DNA), which increases its toxicity when used as a therapeutic agent. Topically applied vitamin D analogs do not cause DNA damage; in fact, they may increase DNA damage repair mechanisms. Although it appears that UVB and vitamin D cause immunosuppression by distinct mechanisms, the end results in terms of Treg and Langerhans cell effects are similar. Therefore, it may be possible to substitute topical vitamin D for UVB phototherapy in some diseases, thereby minimizing the DNA-damaging effects of therapy.

Finally, Schwarz *et al.*'s (2012) observation that vitamin D is instrumental in the development of Tregs, which requires signaling through the vitamin D receptor, and the fact that there are vitamin D receptor polymorphisms that affect the biological response to vitamin D could explain, at least in part, the genetic susceptibility to such immunologically mediated skin diseases, such as psoriasis, melanoma, or non-melanoma skin cancer.

Collectively, Schwarz *et al.* (2012) demonstrate that topical vitamin D can induce antigen-specific Treg cells through a mechanism dependent on Langerhans cells. Although both UVB irradiation and topical vitamin D induce immunosuppression, the ways in which they do so are distinct. The study not only provides new information for the understanding of vitamin D–mediated immunosuppression but also provides a rationale for the potential application of vitamin D or its combination with UVB phototherapy in treating immunodermatological diseases.

CONFLICT OF INTEREST
The authors state no conflict of interest.

REFERENCES

Arnson Y, Amital H, Shoenfeld Y (2007) Vitamin D and autoimmunity: new aetiological and therapeutic considerations. *Ann Rheum Dis* 66:1137–42

Becklund BR, Severson KS, Vang SV *et al.* (2010) UV radiation suppresses experimental autoimmune encephalomyelitis independent of vitamin D production. *Proc Natl Acad Sci USA* 107:6418–23

Bernard JJ, Cowing-Zitron C, Nakatsuji T *et al.* (2012) Ultraviolet radiation damages self noncoding RNA and is detected by TLR3. *Nat Med* 18:1286–90

Elmets CA, Bergstresser PR, Tigelaar RE *et al.* (1983) Analysis of mechanism of unresponsiveness produced by haptens painted on skin exposed to low dose ultraviolet radiation. *J Exp Med* 158:781–94

Hart PH, Gorman S, Finlay-Jones JJ (2011) Modulation of the immune system by UV radiation: more than just the effects of vitamin D?. *Nat Rev Immunol* 11:584–96

Krutmann JT, Elmets CA (eds) (1995) *Photoimmunology*. Blackwell Scientific: Oxford

Palmer MT, Lee YK, Maynard CL *et al.* (2011) Lineage-specific effects of 1,25-dihydroxyvitamin D(3) on the development of effector CD4 T cells. *J Biol Chem* 286:997–1004

Ross AC, Manson JE, Abrams SA *et al.* (2011) The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 96:53–8

Royal Iii W, Mia Y, Li H *et al.* (2009) Peripheral blood regulatory T cell measurements correlate with serum vitamin D levels in patients with multiple sclerosis. *J Neuroimmunol* 213:135–41

Schauber J, Gallo RL (2008) The vitamin D pathway: a new target for control of the skin's immune response?. *Exp Dermatol* 17:633–9

Schwarz A, Navid F, Sparwasser T *et al.* (2012) 1,25-Dihydroxyvitamin D exerts similar immunosuppressive effects as UVR but is dispensable for local UVR-induced immunosuppression. *J Invest Dermatol* 132:2762–9

Schwarz A, Noordegraaf M, Maeda A *et al.* (2010) Langerhans cells are required for UVR-induced immunosuppression. *J Invest Dermatol* 130:1419–27

Schwarz T (2008) 25 years of UV-induced immunosuppression mediated by T cells; from disregarded T suppressor cells to highly respected regulatory T cells. *Photochem Photobiol* 84:10–8

Sreevidya CS, Fukunaga A, Khaskhely NM *et al.* (2010) Agents that reverse UV-induced immune suppression and photocarcinogenesis affect DNA repair. *J Invest Dermatol* 130:1428–37

van der Aar AMG, Sibiryak DS, Bakdash G *et al.* (2011) Vitamin D3 targets epidermal and dermal dendritic cells for induction of distinct regulatory T cells. *J Allergy Clin Immunol* 127:1532–40. e7

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Tyrosinase: A Central Regulatory Protein for Cutaneous Pigmentation

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Cutaneous pigmentation or skin color is the body's natural protection against sun-induced damage. Skin color is determined primarily by melanin, a biopolymer that is synthesized within epidermal melanocytes, packaged in cellular organelles called melanosomes, and then dispersed to neighboring keratinocytes. The process of melanogenesis involves numerous molecules and intracellular pathways that are subject to regulation by endogenous and exogenous factors. Tyrosinase is the central and rate-limiting enzyme in melanin biosynthesis. Therefore, elucidation of the molecules and pathways that regulate tyrosinase levels and activity could identify target areas for the development of compounds to decrease excessive pigmentation on one hand or induce pigmentation on the other. The following commentary will summarize the key regulatory molecules and pathways involved in tyrosinase function.

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Tyrosinase

The human *tyrosinase* gene is about 50 kb long and is composed of 5 exons and 4 introns, whereas tyrosinase messenger RNA (mRNA) is ~2 kb long (genebank access number NM_000372). The nascent chain of the newly synthesized tyrosinase protein is glycosylated, folded in the endoplasmic reticulum (ER), and directed to melanosomes, the organelles residing within melanocytes where melanin synthesis takes place (Park and Yaar, 2012).

Tyrosinase spans the melanosomal outer membrane and has three domains: an inner melanosomal domain that contains the catalytic region of the enzyme, a short melanosomal transmembrane domain, and a cytoplasmic domain. The cytoplasmic domain is composed of a ~30 amino acids, and it is required to properly traffick tyrosinase into melanosomes (Beermann *et al.*, 1995).

Two types of melanin are synthesized within melanosomes: eumelanin and pheomelanin. Eumelanin is dark, brown-black, and insoluble, whereas pheomelanin is light, red-yellow sulfur-containing, and soluble. Tyrosinase is the rate-limiting enzyme in melanin biosynthesis. It catalyzes the conversion of tyrosine through oxidation to L-dihydroxyphenylalanine and requires copper for its activation. Inhibition of this oxidation reaction blocks melanin synthesis (Park and Yaar, 2012). Hydroquinone, the most frequently used drug to treat hyperpigmentation in the United States, is a pseudosubstrate for tyrosinase, thus inhibiting tyrosinase activity.

The final steps in cutaneous pigmentation are the transfer of melanin-containing melanosomes from melanocytes to neighboring keratinocytes, and their dispersion within keratinocytes. Proper dispersal of melanosomes is required for normal skin color and for protective pigmentation. Melanosomes are phagocytosed by keratinocytes, which is facilitated by a seven-transmembrane G protein–coupled receptor called protease activated receptor-2 (PAR-2). PAR-2 is present on the keratinocyte membrane and is activated by serine proteases that cleave the extracellular portion of the receptor, exposing a new segment that acts as a tethered (attached) ligand, increasing keratinocyte phagocytic activity (Nystedt *et al.*, 1994).

Regulating tyrosinase expression and/or activity

A key transcription factor for tyrosinase is the microphthalmia-associated transcription factor (MITF) that, when upregulated, increases tyrosinase expression. MITF has also been intimately linked with melanocyte survival (Park and Yaar, 2012); thus, the effects of MITF extend beyond melanogenesis *per se*. Alpha-melanocyte-stimulating hormone (α-MSH), one of the first recognized positive regulators of mammalian pigmentation, upregulates the expression

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